The survival and vitality of cyanobacteria and algae in fishpond bottom sediments

Přežívání a vitalita sinic a řas v sedimentech rybníka

Petr Hašler, Aloisie Poulíčková & Monika Lysáková

Department of Botany, Faculty of Science, Šlechtitelů 11, CZ-783 71 Olomouc-Holice, Czech Republic.

Abstract

The extraction of sediment cores took place in January 2003 during total ice covering of the investigated fishpond. Sediment cores were cut into 3-cm-thick layers. Sediment samples were cultured and vitality of cyanobateria and algae was studied. Altogether, we identified 49 species of cyanobacteria and algae. Green algae were the most frequent and vital group. Fishpond phytoplankton was dominated by *Planktothrix agardhii* (GOMONT) ANAGNOSTIDIS et KOMÁREK during last 5 years. Cyanobacteria occurred sporadically in sediments.

Introduction

Cyanobacteria and algae form various resting stages during vegetation season, in temperate zone especially in autumn. These surviving stages sink to the bottom, remain in the bottom sediment and wait for better living conditions. Because resting stages can remain viable for a long time, from years to centuries (HARISTON et al. 1995), they can constitute a „seed bank“ for later comeback. In spring, due to sediment disturbances caused by water column mixing and animal activity, they recruit phytoplankton community and germinate. The successful recruitment of benthic stages may be a key in the succession and dominance of cyanobacteria and algae (HANSSON 1996). The successful migration and establishment of alga as a planktonic population can depend on the size of the inoculum and the timing of environmental factors that simultaneously trigger the onset of germination and favour the cell growth. Conditions that are expected to initiate germination include increasing light irradiation, release of nitrogen/phosphorus and sulphide/sulphate from the sediment, seasonal changes in water temperature, etc. (PAERL 1988). Hence, the recruitment from resting stages might be an important process for algal population dynamics. Even though the occurrence of resting stages in sediments had

been described previously (BELMONTE et al. 1997, ELLEGAARD et al. 1994, VAN GEEL 2001, VAN GEEL et al. 1994, VAN GEEL 1998 sec. cit. SOUTH & WHITTICK 1995), their viability has been studied occasionally (MCQUOID et al. 2002, PADAN & COHEN 1982, BAKER 1999, KARLSSON 2003) with a focus on marine environment, namely marine dinoflagellates and diatoms (MCQUOID 2002).

The presented study focuses on vitality, germination and growth of freshwater cyanobacteria and algae from fishpond sediments.

Material and methods

The investigated locality is a small pond situated in a forest garden in the village of Bílá Lhota (coordinates 49°42´35´´N; 16°58´35´´E; altitude 320 m a.s.l.). The total area of the pond is 0.015 ha and maximum depth is 2 m. Shading by the surrounding vegetation is approximately 65%. The eutrophication of the fishpond is probably caused by intensive agricultural practices in the surrounding fields and by sewage-waters from the village of Bílá Lhota. The fishpond has a relatively high conductivity and nutrient concentrations (average annual conductivity 616±44 $\mu S.cm^{-1}$, NO_3^- 1.54±2.15 $mg.l^{-1}$, NH_4^+ 5.10±1.89 $mg.l^{-1}$, TP 2.06±1.35 $mg.l^{-1}$). Phytoplankton is dominated by *Planktothrix agardhii* (up to 90%) with an admixture of euglenophytes (*Euglena acus* EHRENBERG, *E. hemichromata* EHRENBERG, *Trachelomonas* sp. div.) and green algae (*Chlamydomonas* sp. div. *Scenedesmus* sp. div.). The wintering of *Planktothrix agardhii* in the form of hormogonia near the bottom has been described previously (Poulíčková et al. in press.); therefore, their survival in sediments was expected .

As a result of eutrophication, a layer of sediments (90 cm) accumulated in the fishpond during last 43 years (the latest restoration in 1960). The restoration by sediment removal will take place in near future (HAŠLER & POULÍČKOVÁ 2003).

Sediment cores were taken in January 2003 with a core sampler (POKORNÝ & HAUSER 1994). Altogether, 5 sediment cores (70-90 cm thick) were taken. These cores were cut into 3–cm-thick layers. Exact dating of layers is not available, but the history of the fishpond implies that the sediments cannot be older than 43 years. Samples were stored in a cool box before cultivation

The collected samples (100 mg from each layer) were cultured in liquid Zehnder medium (STAUB 1961) in immunological plates (volume 2.5 ml, 4 parallel replicates for each sample) under the temperature regime 18-22°C, photoperiod L/D cycle of 16/8hours, and irradiation of $25\mu mol.m^{-2}.s^{-1}$. During cultivation, all cultures were examined in 7-day intervals under

the inverse microscope directly in immunological plates up to 20 days of cultivation. Quantity of green algae increased during these 3 weeks to such an extent that direct observations became impossible. Further investigations up to 60 days of cultivation were performed by representative sampling of cultivation vessels and examination under LM. The numbers of cells in 1 ml were taken in a counting chamber (Bürker chamber); each time, 400 cells were counted. Cyanobacteria and algae were identified according to STARMACH (1966), HINDÁK (1978), ANAGNOSTIDIS & KOMÁREK (1988), KOMÁREK & ANAGNOSTIDIS (1989).

Results and discussion

Resting stages are thought to be the mechanisms whereby plankton endures in deep, cold, dark, and often anoxic waters of lakes and oceans (BURKHOLDER 1992).

Upper sediment layer can be characterized by significant irradiation and aerobic-anaerobic gradient with the maximum of viable cyanobacteria and algae in first few millimetres PADAN & COHEN (1982). Sediments at fishpond Bílá Lhota were sampled under ice cover; light intensity and oxygen concentrations were low (<1W.m^{-2}; <1 mg.l^{-1} O_2)

Table 1 presents a list of cyanobacteria and algae . Altogether, we identified 49 species (11 cyanobacteria species, 16 diatom species, 1 euglenophyte and 21 species of green algae). Fig 1 presents maximum abundance of the investigated algal groups. Cyanobacteria and flagellates germinated mostly from the upper part of the sediment core (Fig 1). Both green algae and diatoms were observed in the whole sediment core. Since the fishpond phytoplankton was dominated by cyanobacteria, higher proportion of cyanobacteria was expected. On the other hand, the occurrence of flagellate algae (*Chlamydomonas* spp., *Euglena acus*) was a surprise (Fig. 1).

Cyanobacteria

Even though anaerobic conditions can favour the survival of cyanobacteria (PADAN & COHEN 1982), sediment cultures were dominated by green algae; the survival and vitality of cyanobacteria were lower than expected.

Cyanobacteria were represented by genera *Nostoc* and *Anabaena*. Next to initial stages, which were not identified, akinete groups and young growing trichomes were observed (Table I/5,6). The identification of cyanobacteria was impossible before 60 days of cultivation. The role of akinetes in the development of cyanobacterial populations was studied by BAKER (1999). Viable akinetes of *Anabaena circinalis* and *A.flos-aquae* in the surface fine sediment layer of the Murray River were found as an

inoculum of later cyanobacterial growth. Differences in akinete germination had been recorded previously by GIBSON & SMITH (1982). Some of them germinate immediately (e.g. *Anabaena*, *Aphanizomenon*, *Gleotrichia*) while other can germinate in the following years. Culture conditions, for instance phosphate concentration, can influence akinete germination (*A. cylindrica*; GIBSON & SMITH (1982). Although cyanobacteria are successfully cultured in used Zehnder medium in culture collections (LUKAVSKÝ et al. 1992), the phosphorus concentration is probably lower than in natural conditions of the investigated fishpond (TP in Zehnder medium = 16.54 $\mu g.l^{-1}$; fishpond Bílá Lhota = annual average of TP concentrations 2.06 $mg.l^{-1}$)

However phytoplankton was dominated by *P. agardhii* throughout last five years and its wintering in the form of hormogonia was observed near the bottom, germination of *P.agardhii* was observed rarely (Table I/2) at the end of the experiment (60 days of cultivation). GIBSON & FITZSIMONS 1982 found the wintering of other oscillatorean cyanobacteria in the water column to be their main survival strategy. The germination of *Oscillatoria* sp. (layer 3) and *P. agardhii* (layer 1,9) from sediment samples was observed; therefore, we suppose that some resting bottom stages of these cyanobacteria probably exist.

Diatoms

For some algae, physiological resting stages (as opposed to resting spores or cysts, which are easily discernible with light microscopy) were observed. SICKO-GOAD et al. (1989) identified resting cells in 18 diatom species collected from aphotic sediments in the Laurentian Great Lakes. These cells had all the cytoplasm condensed into one dense mass, which usually laid in the centre of the frustule. They found that these resting cells could rejuvenate within 2 hours of re-illumination.

Although the direct examination of sediment samples before the cultivation established only the presence of empty diatom frustules, diatoms were observed among first growing algae, already the 7th day of experiment. Small *Cyclotella* sp. bunches among green algae occurred first. These small diatoms (3 - 5 μm in diameter) were replaced later (after the 14th day) by pennate diatoms, e.g. *Navicula*, *Pinnularia* and *Gomphonema*. After 45 days of cultivation, the majority of viable diatoms disappeared. All frustules were thin, with indistinct stria pattern due to poor silicification. The influence of light intensity, photoperiod and temperature on diatom germination has been studied previously by MCQUOID et al. (2002). They observed the germination even after the 1st day of cultivation under optimum conditions (MCQUOID & HOBSON 1995, 1996).

Green algae and euglenophytes

Under unfavourable conditions, particularly desiccation, many Chlorophyceae produce thick-walled resting cells (COLEMAN 1983 sec. cit. SOUTH & WHITTICK 1995). LEMBI (1980) sec. cit. SOUTH & WHITTICK (1995) recorded similar resting cells in unicellular volvocalean green algae Akinetes are modified vegetative cells with a thickened wall. Hypnospores and hypnozygotes also have thickened walls, but these are produced de novo by protoplasts which had separated earlier from the walls of parental cells. In laboratory, such cells may remain viable for periods exceeding 20 years (COLEMAN 1983 sec. cit. SOUTH & WHITTICK 1995).

The most frequent green algae were the representatives of genus *Scenedesmus.* Tables 2/6, 7 present the development of their coenobia from small resting cells. Similar stages had been previously recorded in *Scenedesmus abundans* by HINDÁK (personal communication). Flagellates were observed in the upper part of sediment, initially as pallmeloid stages (Table III/6), later were identified as representatives of genus *Chlamydomonas* (Table III/3,5). SUZUKI & JOHNSON (2002) studied the photoperiodic control of germination in the unicellular *Chlamydomonas.* They demonstrated that the occurrence of zygospore germination of this alga is a genuine photoperiodic response. Germination efficiency is enhanced in long days (15/9, 12/12, 11/13) compared to short days (9/15, 8/16).

Acknowledgement

This work was supported by the project FRVŠ 63/2003 of the Ministry of Education of the Czech Republic GAČR No. 522/03/0323. The authors express thanks to Mr. P. Hekele, the manager of the forest garden Bílá Lhota, for his co-operation.

References

ANAGNOSTIDIS, K. & KOMÁREK, J. (1988): Modern approach to the classification system of cyanophytes 3. Oscillatoriales. – Algological Studies 50–53: 327–472.

BAKER, P. D. (1999): Role of akinetes in the development of cyanobacterial populations in the Lower Murray River, Australia. – Mar. Freshwater Res. 50: 265–279.

BELMONTE, G., MIGLIETTA, A., RUBINO, F. & BOERO, F. (1997): Morphological convergence of resting stages of planktonic organisms: a review. – Hydrobiologia 355: 159–165.

BURKHOLDER, J. M. (1992): Phytoplankton and episodic suspended sediment loading: Phosphate partitioning and mechanisms for survival. – Limnol. Oceanogr. 37: 974–988.

ELLEGAARD, M., CHRITSENSEN, N. F. & MOESTRUP, Ø. (1994): Dinoflagellate cysts from Recent Danish marine sediments. – Eur.J.Phycol. 29: 183–194.

GIBSON, C. E. & FITZSIMONS, A. G. (1982): Periodicity and morphology of planktonic blue–green algae in an unstratified lake. – Internat. Rev. ges. Hydrobiol. 67: 459–476.

GIBSON, C. E. & SMITH, R. V. (1982): Freshwater Plankton. – In: CARR, N.G. & WHITTON, B. A. (eds): The Biology of Cyanobacteria, p. 463–490, Blackwell Scientific Publications, Oxford.

HANSSON, L–A. (1996): Algal recruitment from lake sediments in relation to grazing, sinking and dominance patterns in the phytoplankton community. – Limnology and Oceanography 41(6): 1312–1323.

HARISTON, N. G., VAN BRUNT, R. A., KEARNS, C. M. & ENGSTROM, D. R. (1995): Age and survioship of diapausing eggs in a sediment egg bank. – Ecology 76: 1706–1711.

HAŠLER, P. & POULÍČKOVÁ, A. (2003): Diurnal changes in vertical distribution and morphology of natural population of *Planktothrix agardhii* (GOM.) ANAGN. & KOM. (Cyanophyta). – Hydrobiologia 506–509: 195–201.

KARLSSON, I. (2003): Benthic growth of Gloeotrichia echinulata Cyanobacteria. – Hydrobiologia, in press.

KOMÁREK, J. & ANAGNOSTIDIS, K. (1989): Modern approach to the classification system of cyanophytes 4. Nostocales. – Algological Studies 56: 247–345.

LUKAVSKÝ, J., CEPÁK, V., KOMÁREK, J., KAŠPÁRKOVÁ, M. & TAKÁČOVÁ, M. (1992): Catalogue of algal and cyanobacterial strains of Culture Collection of Autotrophic Organisms at Trebon. – Algological Studies 63: 59–112.

MCQUOID, M. R., GODHE, A. & NORDBERG, K. (2002): Viability of phytoplankton resting stages in the sediments of a costal Swedish fjord. – Eur.J.Phycol. 37: 191–201.

MCQUOID, M. R. & HOBSON, L. A. (1995): Importance of resting stages in diatom seasonal succession. – J.Phycol. 31: 44–50.

MCQUOID, M. R. & HOBSON, L. A. (1996): Diatom resting stages. – J.Phycol. 32: 889–902.

PADAN, E. & COHEN, Y. (1982): Anoxygenic Photosynthesis. – In: CARR, N.G. & WHITTON, B.A. (eds): The Biology of Cyanobacteria, p. 215–236, Blackwell Scientific Publications, Oxford.

PAERL, H. W. (1988): Nuisance phytoplankton blooms in coastal, estuarine and inland waters. – Limnology and Oceanography 33: 823–847.

POKORNÝ, J. & HAUSER, V. (1996): Restoration of lakes by sediment removal – fishpond Vajgar, Czech Republic. – In: EISELTOVÁ, M. (ed.): Restoration of lake ecosystems – a holistic approach, 182 pp., Nature Conservation Bureau, Berkshire.

POULÍČKOVÁ, A., HAŠLER, P. & KITNER, M. (in press): Annual cycle of *Planktothrix agardhii* (GOM.) ANAGN. & KOM. nature population. – Int. Rev. Hydrobiol.

SICKO–GOAD, L., STOERMER, E. F. & KOCIOLEK, J. P. (1989): Diatom resting cell rejuvenation and formation: Time course, species records and distribution. – J. Plankton Res. 11: 375–389.

SOUTH, G. R. & WHITTICK, A. (1995): Introduction to phycology. – 293 pp., Blackwell Scientific Publications, Oxford.

STARMACH, K. (1966): Cyanophyta–sinice, Glaucophyta – glaukofity. – In: STARMACH, K. (ed.): Flora slodkow.Polski 2, 753 pp., PAN, Pañstw.Wyd. Nauk, Warszawa.

STAUB, R. (1961): Ernährungsphysiologisch-autökologische Untersuchungen an der planktonischen Blaualge *Oscillatoria rubescens* DC. – Schweiz. Z . Hydrol. 23: 82–198.

SUZUKI, L. & JOHNSON, C. H. (2002): Photoperiodic control of germination in the unicell *Chlamydomonas*. – Naturwissenschaften 89: 214–220.

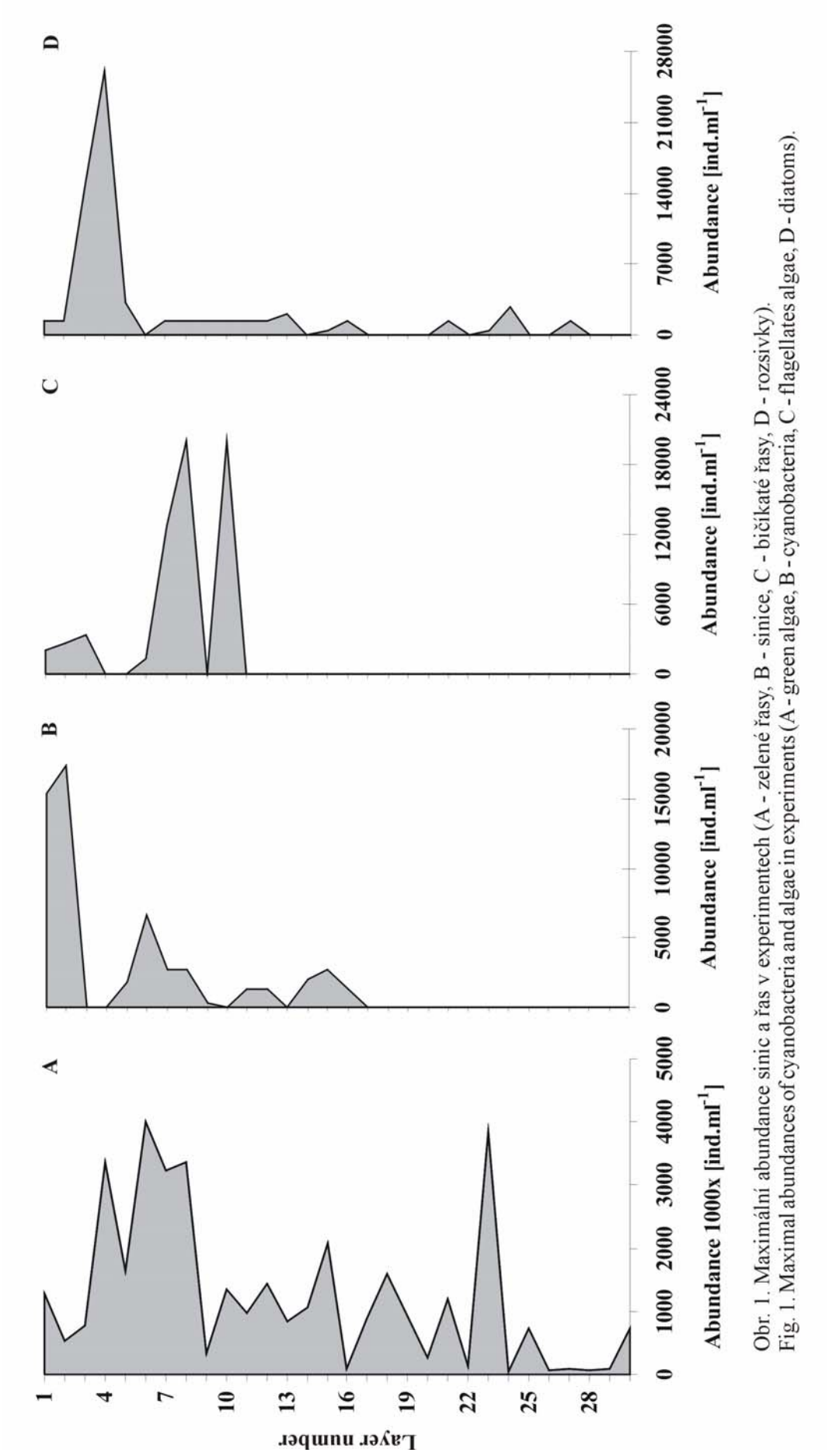
VAN GEEL, B. (2001): Non–pollen palynomorphs. – In: SMOL, J.P., BIRKS, J.B. & W.M. LAST (eds): Tracking Environmental Change Using Lake Sediments. Volume3: Terrrestrial, Algal and Siliceous Indicators, p. 99–119, Kluwer Academic Publishers, Dordrecht.

VAN GEEL, B., MUR, L. R., RALSKA–JASIEWICZOWA, M & GOSLAR, T. (1994): Fossil akinetes of Aphanizomenon and Anabaena as indicators for medieval phosphate–eutrophication of Lake Gosciaz (Central Poland). – Review of Palaeobotany and Palynology 83: 97–105.

Table 1: List of cyanobacteria and algae found in sediment of fishpond Bílá Lhota

Species	Found in layer number:
Cyanobacteria	
Anabaena flos-aquae BRÉBISSON ex BORNET et FLAHAULT	12
Anabaena inaequalis BORNET et FLAHAULT	2, 7, 11
Anabaena cf. *sphaerica* BORNET et FLAHAULT	8, 12, 14
Anabaena sp.	5
Anabaena sp.	9
Nostoc linckia (ROTH) BORNET ET FLAHAULT	2, 6, 9
Nostoc sp.	7
Nostoc sp.	11
Nostoc sp.	15
Oscillatoria sp.	3
Planktothrix agardhii (GOMONT) ANAGNOSTIDIS et KOMÁREK	1, 9
Bacillariophyceae	
Cocconeis placentula EHRENBERG	7, 12
Cyclotella atomus HUSTEDT	1-3, 5, 7-10, 13, 15
Cyclotella cf. *meneghiniana* KÜTZING	27
Cyclotella sp.	4
Fragilaria sp.	7
Gomphonema parvulum (KÜTZING) KÜTZING	7
Navicula rhynchocephala KÜTZING	7, 9, 10
Navicula sp.	11
Navicula sp.	12
Navicula sp.	15
Nitzschia communis RABENHORST	7
Nitzschia cf. obtusa	11
Pinnularia cf. *viridis* (NITZSCH) EHRENBERG	13
Pinnularia sp.	10
Pinnularia sp.	21
Synedra ulna (NITZSCH) EHRENBERG	3

Euglenophyta	
Euglena acus EHRENBERG	9, 11
Chlorophyta	
Actinastrum hantzschii LANGERHEIM	4
Chlamydomonas debaryana GOROSH.	3, 7, 8, 10
Chlamydomonas sp.	1
Chlamydomonas sp.	2
Chlamydomonas sp.	4
Chlamydomonas sp.	6
Chlamydomonas sp.	8
Chlamydomonas sp.	10
Chlorella homosphaera SKUJA	1, 6, 10, 14, 18
Chlorella luteoviridis CHODAT	3
Chlorella vulgaris BEIJERINCK	2, 4, 5, 8-11, 20, 25, 30
Chlorogonium euchlorum EHRENBERG	
Monoraphidium contortum (THURET in BRÉBISSON) KOMÁRKOVÁ-LEGNEROVÁ	2, 23
Monoraphidium convolutum (CORDA) KOMÁRKOVÁ-LEGNEROVÁ	7, 25
Monoraphidium griffithii (BERKELEY) KOMÁRKOVÁ-LEGNEROVÁ	1-5
Scenedesmus abundans (LANGERHEIM) CHODAT	1-5, 7-9, 11-14, 16, 17
Scenedesmus acuminatus (LANGERHEIM) CHODAT	1, 3, 30
Scenedesmus alternans REINSCH	6, 20
Scenedesmus ecornis (RALFS) CHODAT	1-3, 6
Scenedesmus intermedius CHODAT	4, 8, 12, 15, 17, 23
Scenedesmus sp.	16



Obr. 1. Maximální abundance sinic a řas v experimentech (A - zelené řasy, B - sinice, C - bičíkaté řasy, D - rozsivky).
Fig. 1. Maximal abundances of cyanobacteria and algae in experiments (A - green algae, B - cyanobacteria, C - flagellates algae, D - diatoms).

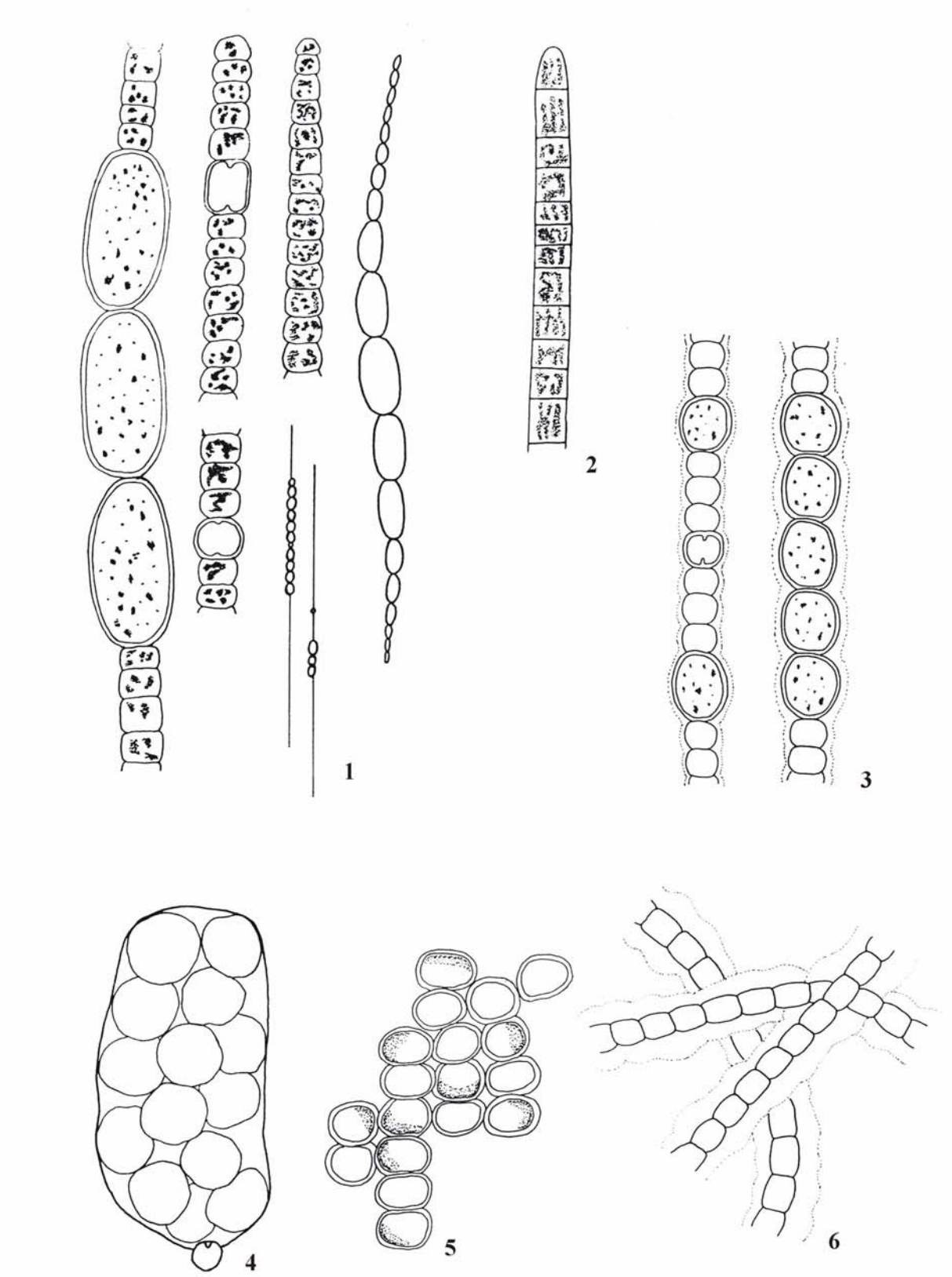


Fig. 2: 1 - *Anabaena inaequalis*, 2 - *Planktothrix agardhii*, 3 - *Nostoc linckia*, 4 - *N. linckia* (hormogonium), 5 - *N. linckia* (akinetes formation), 6 - *N. linckia* (young growing filaments)

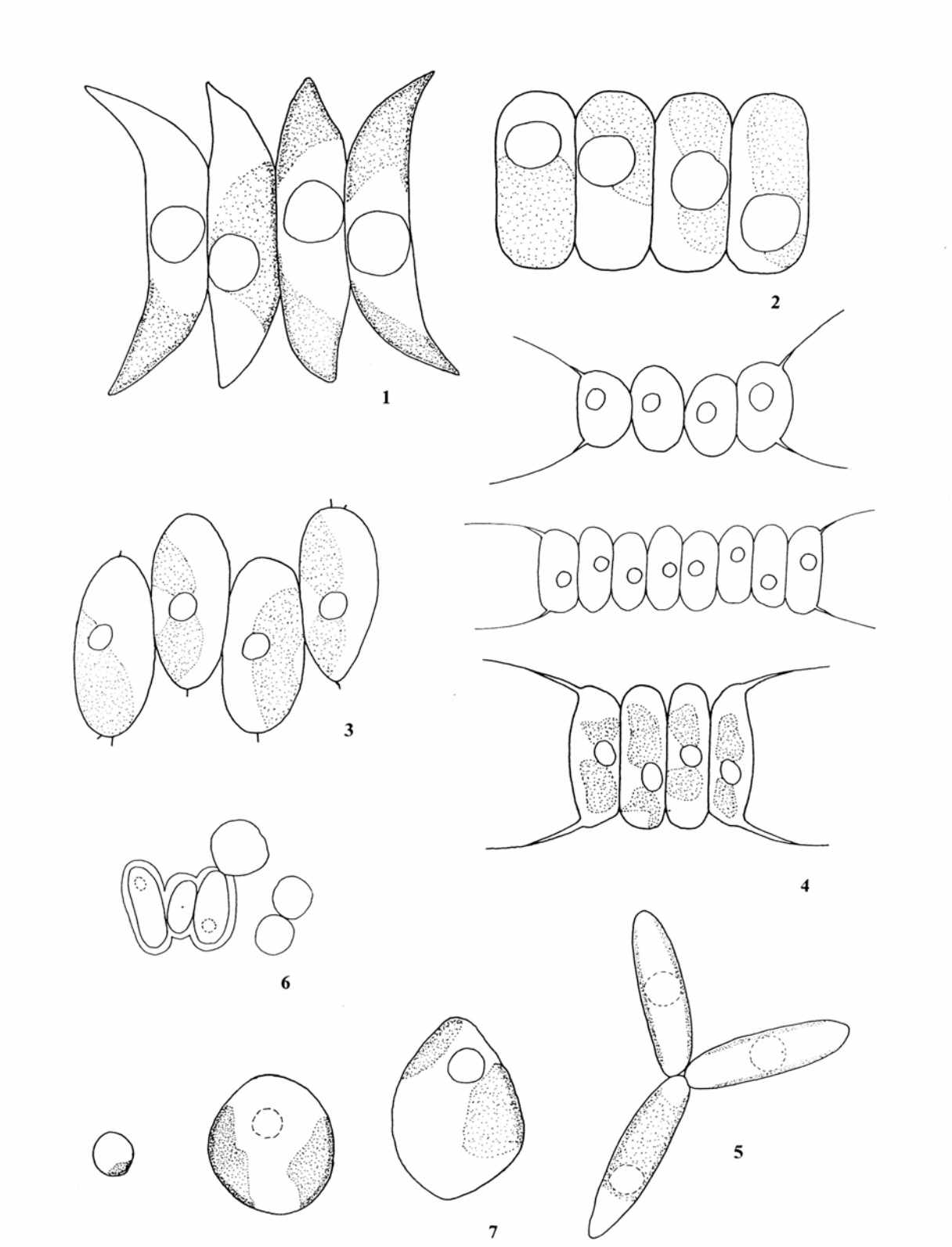


Fig. 3: 1 - *Scenedesmus acutus*, 2 - *S. linearis*, 3 - *S. denticulatus*, 4 - *S. quadricauda*, 5 - *Actinastrum hantzschii*, 6 - 7 - developmental stages of *Scenedesmus*

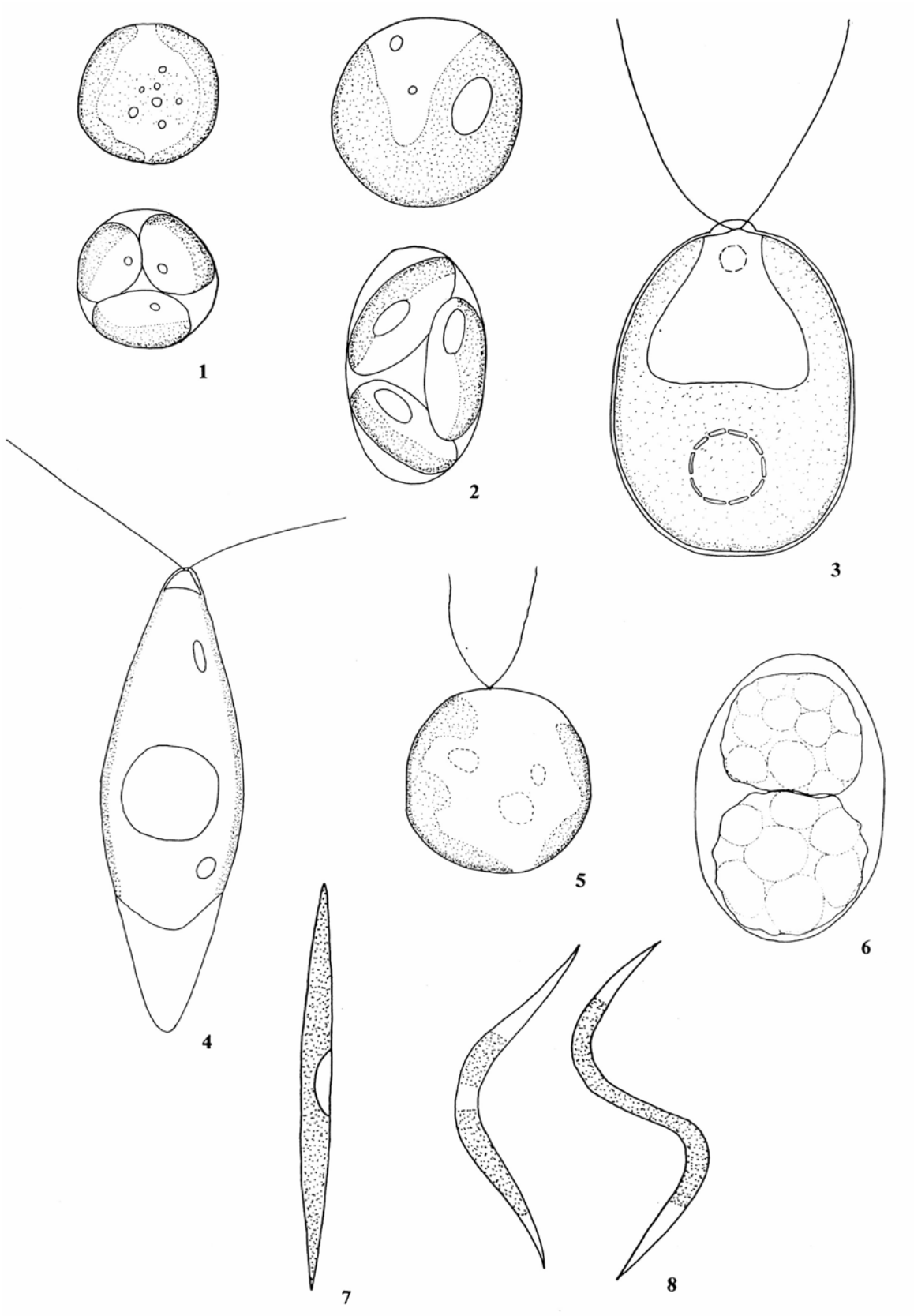


Fig. 4: 1 - *Chlorella homosphaera*, 2 - *Ch. vulgaris*, 3 - *Chlamydomonas debaryana*, 4 - *Chlorogonium euchlorum*, 5 - *Chlamydomonas* sp., 6 - *Chlamydomonas* (palmelloid stage), 7 - *Monoraphidium griffithi*, 8 - *M. contortum*